

## PHYTOCHEMICAL SCREENING AND ANTI-*TRICHOSPORON* ACTIVITY OF *PLUMBAGO ZEYLANICA* LINN

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Received: 31 May 2014 Revised and Accepted: 01 Aug 2014

### ABSTRACT

**Objective:** To evaluate the anti-*Trichosporon* activity of a medicinal plant, *Plumbago zeylanica* Linn and to find out the phytochemicals responsible for this activity with the use of four solvent extracts; methanol, ethanol, acetone and chloroform.

**Methods:** To evaluate the antifungal activity, two methods were used. Agar well diffusion assay to determine the diameter of zone of inhibition (in mm) and MIC (in mg/ml) was determined through Tube dilution broth assay. Phytochemical screening for preliminary analysis of secondary metabolites was done through chemical methods.

**Results:** *Trichosporon asahii* was found to be most sensitive to methanol extract of *P. zeylanica* with inhibition zone of 35 mm and *T. inkin* with 30 mm zone of inhibition. MIC results also showed that the methanol extract was most effective in lowest concentration against *T. asahii* with MIC at 6.2 mg/ml and inhibited *T. inkin* at 25 mg/ml. Phytochemical screening of plant extracts revealed the presence of certain phyto-constituents like alkaloids, steroids, flavonoids, phenols, glycosides, tannins etc.

**Conclusion:** The present study proved the sensitivity of the yeast *Trichosporon* spp. to different extracts of *P. zeylanica*. *T. asahii* was found to be more sensitive towards the extracts. Methanol extract proved to be most effective compared to ethanol, acetone and chloroform as it gave the maximum zones and lowest MIC result. Phytochemical analysis proved the presence of certain phytochemicals which was responsible for their antifungal activity.

**Keywords:** *Plumbago zeylanica*, Extract, Antifungal activity, MIC, Phytochemical analysis.

### INTRODUCTION

In humans, fungal infections range from superficial to deeply invasive or disseminated and have increased dramatically in recent years. The treatment of mycoses has lagged behind bacterial chemotherapy and fewer antifungals than antibacterial substances are available [1].

*Trichosporon* species is a medically important genus which is the major causative agent of White and Black Piedra and also the causative agent of disseminated infections in immune-compromised hosts. *Trichosporon asahii* and *T. inkin* behave as opportunistic pathogens, particularly in immune-suppressed patients where they can have potentially life threatening consequences. In the immune-compromised person, dissemination can take place, causing pruritic or necrotic cutaneous papules and nodules due to *Trichosporon* infection. This pathogenic species is a saprophyte found in the environment (soil, water and plants) as well as in the skin and excretion of mammals and humans [2]. More recently, these have been recognized as an opportunistic pathogen that can cause a potentially fatal systemic infection in immune compromised hosts with Fungemia, pulmonary infiltrates, renal damage and pustular, nodular, purpuric or necrotic skin lesions, strengthening the recommendations of dermatologists for prompt recognition and adequate treatment of the disease [3, 4].

Natural products derived from plants offer a new source of biological that may have a great impact on infectious diseases and overall human health [5, 6]. The medicinal uses of plants are spread all over the world as folk medicine. Based on this wide use of plants for medicinal purposes, over than 1500 plants have been scientifically studied regarding their phytochemical profile and pharmacological properties. Plants have been used traditionally for many centuries as abortifacients, contraceptives, for menstrual regulation, fertility control as well as for the treatment of ailments of both microbial and non-microbial origins [7].

Recently some higher plant products have attracted the attention of microbiologists to search for some phytochemicals for their exploitation as anti-microbial. Such plant products would be biodegradable and safe to human health [8].

The present study was carried out to determine the effectiveness of extracts of *Plumbago zeylanica* Linn. Against *Trichosporon* species which is an opportunistic pathogen. The leaves of *P. zeylanica* are widely used medicinally in India. Traditionally, *P. zeylanica* is believed to kill intestinal parasites, and it is used clinically to treat rheumatism, intestinal parasites, anemia due to "stagnant blood", external and internal trauma, toxic swelling and malignant furunculosis scabies [9]. Pharmacological studies have indicated that *P. zeylanica* extract has anti-plasmodial [10], antimicrobial [11] antifungal [12] and anti-inflammatory activity [13].

### MATERIALS AND METHODS

#### Collection, identification and extraction methods

Fresh leaves of the plant were collected from the various regions of Dehradun city. The taxonomic identity of the plants was confirmed by Department of Botany, Forest Research Institute, Dehradun. Leaves were washed under running tap water, air dried and then homogenized to fine powder and stored in air tight bottles. The air dried and powdered plant material (100 g) was extracted with 200 ml of each solvent (Methanol, ethanol, acetone and chloroform), kept on a rotary shaker for 24 hrs. Thereafter it was filtered and centrifuged at 5000 x g for 15 min. The supernatant was collected and evaporated to dryness to give the crude dried extract.

#### Fungal cultures

The test fungal strains investigated include two *Trichosporon* species which were procured from Microbial Type Culture Collection Centre (MTCC), Chandigarh and National Collection of Yeast Cultures (NCYC) Norwich, United Kingdom. They were maintained on

selective media Yeast Malt Agar slants and plates at an optimum temperature of 25°C and experiments were carried out on Sabouraud Dextrose Agar. The two yeast cultures used were as follows:

1. *Trichosporon asahii* (MTCC No. 6179)
2. *Trichosporon inkin* (NCYC No. 2515)

### Antifungal assays

#### Agar well diffusion assay

Preliminary analysis of antifungal activity was conducted using Agar Well Diffusion Assay [14]. Fungal inoculum was prepared in saline solution and incubated for 1 hour. Molten Sabouraud Dextrose Agar (SDA) was added with 1 ml of fungal inoculum into presterilized petriplates. After solidification, wells of 6 mm diameter were punctured in the culture medium using a sterile cork borer. A fixed volume (100 µl) of respective crude extract prepared in 5% Dimethyl Sulphoxide (DMSO) was loaded in the well using sterilized micropipettes. Plates were incubated for 2 days at 25°C and zone of inhibition (in mm) of different extracts was determined after 48 hrs. Sterile 5% aqueous DMSO was used as negative control while Ketoconazole and Nystatin B were used as the positive control. All experiments were carried out in triplicates.

#### Tube dilution broth assay

MIC of the extracts against the test fungi was determined using the Broth Dilution Method [15]. Various concentrations (600-1.55 mg/ml) of the extracts were prepared by dissolving extracts in 5% DMSO. 1 ml of the plant extract (100 mg/ml) was added to 1 ml of Sabouraud Dextrose broth in test tubes and subsequent concentrations were prepared by using serial dilution technique. 100 µl fungal culture prepared in saline water were inoculated into each test tube and mixed thoroughly on a vortex mixer. The test tubes were then incubated at 25°C for 2 days. The MIC values were determined macroscopically after 48 hrs of incubation in comparison with the growth and sterility controls [16]. Ketoconazole and Nystatin B was used as positive controls. All the experiments were performed in triplicate.

#### Phytochemical screening of the plant extracts

The Phytochemical components of the medicinal plants were screened using the following methods [17, 18].

- (a) Saponins: 25 g each of the powdered samples were boiled in 25 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of an emulsion.
- (b) Steroids: 2 ml of acetic anhydride were added to 0.5g ethanol extract of each sample with the addition of 2 ml H<sub>2</sub>SO<sub>4</sub>. A color change from violet to blue or green indicates the presence of steroids.

(c) Flavonoids: A portion of the powdered plant samples were separately heated with 10 ml of ethyl acetate in a water bath for 3 min. The mixtures were filtered and 4 ml of each filtrate was shaken with 1 ml of dilute ammonia solution. A yellow color observation indicates the presence of flavonoids.

(d) Tannins: 0.5g of each powdered samples were boiled in 20 ml of water in a test tube and then filtered. Few drops of 0.1 % ferric chloride was added and observed for brownish green or blue black colour.

(e) Total Phenol: 2g each of the samples were defatted with 1 ml of diethyl ether using a soxhlet apparatus for 2 hrs. Fat free samples were boiled with 50 ml of ether for the extraction of the phenolic components for 15 minutes. 5 ml of the extracts were pipette into 50 ml flask and then 10 ml distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 minutes for color development.

(f) Alkaloid: 5g of each sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 48 hrs. After filtration, the extracts were concentrated on a water bath to ¼ of the original volume. Concentrated ammonium hydroxide was added in drops to the extract until the precipitation was collected, washed with dilute ammonium hydroxide and then filtered. The residue obtained is the alkaloid, and was dried and weighed.

(g) Terpenoids: To 0.5 g of extract, was added 2 ml of chloroform. 3 ml of concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

#### Statistical analysis

The inhibitory zones of plant extracts were expressed as the Mean ± Standard deviation at P < 0.05.

#### Results

Results of antifungal activity through Agar well diffusion assay is shown in **Table 1**.

Table 1. Clearly shows that methanol extract of leaves of the plant were more effective as compared to other solvents as it inhibited the fungal growth with maximum zone of inhibition (35 mm) against *T. asahii* and *T. inkin* (30 mm). The solvents exhibited the overall effectiveness in the order Methanol > Ethanol > Acetone > Chloroform. Comparison with positive controls proved that methanol and ethanol solvent extracts showed good anti-yeast activity against both *Trichosporon* spp.

Tube dilution broth assay revealed the lowest concentrations at which the solvent extracts inhibited the growth of the test fungi. (Table 2).

**Table 1: Agar well diffusion assay of different solvent extracts of *Plumbago zeylanica* Linn. leaves**

Yeast species	Zone of inhibition (mm)					
	Solvent extracts				Positive controls (mm)	
	M	E	Ch	A	Ke	Ny
<i>Trichosporon asahii</i>	35±0.5	20±0.1	18±0.5	16±0.5	22±0.0	25±0.0
<i>Trichosporon inkin</i>	30±0.6	25±0.1	20±0.5	23±0.6	25±0.0	15±0.0

M-Methanol; E-Ethanol; Ch-Chloroform; A-Acetone; Ke-Ketoconazole; Ny-Nystatin

**Table 2: MIC of different solvent extracts of *Plumbago zeylanica* Linn. leaves**

Yeast species	Minimum inhibitory concentration (mg/ml)					
	Solvent extracts				Positive control	
	M	E	Ch	Ac	Ke	Ny
<i>T. asahii</i>	6.2±0.6	100±0.3	100±0.6	200±1.0	50±0.0	200±0.0
<i>T. inkin</i>	25±0.5	50±0.6	100±0.5	100±0.5	50±0.0	200±0.0

M: Methanol; E: Ethanol; Ch: Chloroform; Ac: Aetone; Ke: Ketoconazole; Ny: Nystatin

This assay shows that methanol extract of the plant inhibited the growth of the yeast at the lowest MIC (6.2 mg/ml) against *T. asahii* and *T. inkin* (25 mg/ml). Other extracts exhibited MIC in the range of 50-200 mg/ml.

**Table 3: Phytochemical screening of the extracts of *P. zeylanica* Linn.**

<i>P. zeylanica</i>	Phenols	Terpenoids	Alkaloids	Flavonoids	Steroids	Tannins	Saponins
Methanol	+	-	+	+	-	+	+
Ethanol	+	-	+	+	-	+	+
Chloroform	-	+	-	-	+	-	-
Acetone	-	-	+	+	+	+	-

Phytochemical screening of plant extracts revealed the presence of certain phyto-constituents like alkaloids, steroids, flavonoids, phenols, glycosides, tannins etc. (Table 3.)

#### DISCUSSION

*Trichosporon* species are long known as the cause of superficial infections such as white piedra, a distal infection of the hair shaft [19], the genus is now the second most commonly reported cause of disseminated yeast infections in humans [20]. This species is increasingly recognized as a cause of systemic illness in immunocompromised patients [21]. Medicinal plants have always provided a stable source for medicines. Not only the herbs themselves but certain plant derived compounds have served as lead molecules for further chemical modulation and natural products still continue to play a highly significant role in drug discovery and development process [22].

Plant extracts have been used traditionally to treat a number of infectious diseases including those caused by bacteria, fungi, protozoa and viruses [23, 24, 25]. A number of reports are available for *in vitro* and *in vivo* efficacy of plant extracts against plant and human pathogens causing fungal infection [26].

Antimicrobial activity and phytochemical screening of crude extracts of *Plumbago zeylanica* Linn. Against a variety of microorganisms were also reported by other workers [27, 28] but no reports of *P. zeylanica* activity against *Trichosporon* species was found to be published.

The phytochemical screening of the plant studied showed that the leaves of *P. zeylanica* were rich in most of the secondary metabolites analyzed using different solvents as shown in Table 3. These chemical constituents were reported to show medicinal activity as well as exhibiting physiological activity [29]. Some of these plants metabolites were found to be absent in some of the solvent extracts used.

Thus the study confirms that *Plumbago zeylanica* can be used as an important source of antifungal compounds that may provide renewable source of useful antifungal drugs against *Trichosporon* infections in humans.

#### CONFLICT OF INTERESTS

Declared None

#### ACKNOWLEDGEMENT

Thanks are due to Head of Botany Department, HNB Garhwal University, Srinagar, for providing us the lab facility; NCYC and MTCC for the procurement of yeast cultures and to HOD, Department of Botany and Forest Research Institute, Dehradun for the Identification of Plant species.

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